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Title: Faecal calprotectin in treated and untreated children with coeliac disease and juvenile idiopathic arthritis

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Word count: 1,200

Keywords: calprotectin, juvenile idiopathic arthritis, coeliac disease

Conflicts of interest and source of funding

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ABSTRACT

This study aimed to provide evidence on whether children at risk of gastrointestinal inflammation **have increased measurements of** faecal calprotectin (FC). Faecal calprotectin was measured **in 232 children**; 55 children (n=11 treatment naïve) with juvenile idiopathic arthritis (JIA), 63 with coeliac disease (CD); 17 with new diagnosis before and after treatment on gluten free diet and **114** controls. None of the treatment-naïve children with JIA had raised FC. Four JIA patients on treatment had a raised FC but in all cases a repeat test was normal. In newly diagnosed CD patients, the median (IQR) FC was higher 36.4 (26-61) than in controls 25.0 (23-41) mg/kg ($p=0.045$) but this significantly decreased 25 (25-25) mg/kg ($p=0.012$) after six months on gluten free diet. **Random measurements of FC are not raised in children with** JIA or CD. A significant elevation of FC in these groups is not explained by their diagnosis and therefore needs further investigation.

What is known

- Faecal calprotectin is an established biomarker for the differential diagnosis of inflammatory bowel disease

What is new

- Faecal calprotectin, **in spot samples**, is not raised in children with treated or untreated juvenile idiopathic arthritis or coeliac disease
- Repeatedly elevated measurements of faecal calprotectin in these groups are likely to be clinically significant and should prompt initiating investigations for inflammatory bowel disease.

INTRODUCTION

Faecal calprotectin (FC) has become an established biomarker for the differential diagnosis of inflammatory bowel disease (IBD) (1). National guidelines advocate the use of FC testing in clinical practice, reducing the number of unnecessary referrals and expensive diagnostic procedures in specialist centres (2). As the number of assessments for colonic inflammation is likely to increase in individuals with other gastrointestinal diseases, or in individuals at risk of developing IBD, evidence is required as to whether the clinical benefit of such practice balances the associated analytical costs and resource allocation.

This study aimed to provide preliminary evidence on whether spot measurements of FC are raised in certain groups of children at risk of gastrointestinal inflammation. We measured FC in children with different types of juvenile idiopathic arthritis (JIA) and in children with coeliac disease (CD) prior to and during treatment with gluten free diet (GFD).

METHODS

Participants

Faecal calprotectin concentration was measured in 242 samples in the different groups of children. These were samples collected from:

- a. Seventeen children with newly diagnosed CD, before and after (n=13) six month treatment on GFD. Children with CD disease were diagnosed according to the European Society of Paediatric Gastroenterology, Hepatology and Nutrition diagnostic recommendations. All children had raised IgA tissue Transglutaminase antibodies and endomysial testing suggestive of CD (3).
- b. Forty six children with longstanding CD on treatment with GFD diet for longer than 12 months [disease duration, median (IQR): 3.1 (1.7 to 7.3) years].
- c. Eleven children with newly diagnosed, treatment-naïve JIA and 44 others on disease modifying treatment [disease duration, median (IQR): 4.1 (2.0 to 7.2) years] including biologic therapies. The JIA patients consisted of 7 (13%) with psoriatic arthritis, 13 (24%) with enthesitis related arthritis, and the remaining 35 with other types of arthritis. Nine of the forty patients with available measurements were positive for Human Leukocyte Antigen B27 (HLA-B27). The number (%) of children on treatment with methotrexate, biologics, oral steroids and steroidal injections were 27 (61%), 16 (36%), 4 (9%) and 9 (20%) respectively. Six (14%) of the children were using non-steroidal anti-inflammatory drugs and two (5%) were on azathioprine. None of these children reported chronic gastrointestinal symptoms or was under investigations for IBD. Four of the JIA children were on modifying treatment and one from the treatment naïve JIA patients had a parent with IBD (Table 1).
- d. One hundred and fourteen healthy children with no previous history of gastrointestinal disorders acted as reference range. Of these, 22 were siblings of patients with CD with negative IgA tissue Transglutaminase antibodies.

Measurement of calprotectin

Faecal calprotectin concentration was measured in samples using the CALPROLAB™ Calprotectin ELISA (CALPRO, Norway) kit(4). The lower detection limit of the assay is 25 mg/kg. In children with CD, gastrointestinal symptoms were assessed with the validated PedQL™ Gastrointestinal Symptoms Scale.

Statistical analysis

Differences in FC between groups were compared with Mann-Whitney and Kruskal-Wallis tests or chi squared test for FC classes (i.e. normal vs raised). Correlations between FC and continuous parameters were explored with Spearman rank correlation. The FC results were classified into three groups, between 25 to 50 mg/kg which is the manufacturer's reference threshold, between 50 to 100 mg/kg and higher than 100 mg/kg as the latter has been recommended by the National Institute for Health and Care Excellence in UK as a cutoff with improved diagnostic accuracy(2). Statistical analysis was performed with Minitab 16 (Minitab Ltd, Coventry, UK) and MedCalc 15.6.

Ethical approval

All studies were approved by the West of Scotland, Research Ethics Committee and the Research and Development office at NHS Greater Glasgow and Clyde. Every participant or their careers, received written information prior to giving signed consent.

RESULTS

Children with JIA

None of the newly diagnosed, treatment-naïve children with JIA had a FC higher than 100 mg/kg (Figure 1), with the large majority of participants (64%) presenting values below the detection limit of the assay (i.e. 25 mg/kg). Of the JIA patients on disease modifying treatment, four had FC concentration above 100 mg/kg. All of these four children provided a follow up sample for a repeat FC and in all cases this was deemed normal (FC < 100 mg/kg). There was no significant difference in median FC concentrations between the enthesitis related arthritis, psoriatic arthritis and other types of JIA (Table 1). None of these children had a FC concentration higher than 100 mg/kg. There was no significant difference in the concentration of FC between users and non-users of non-steroidal anti-inflammatory drugs [median (IQR) mg/kg, users vs. non-users; 25 (25-35) vs. 27 (25-46), $p=0.177$].

Children with CD

There was no significant difference in the median concentration (Table 1) or proportion (Figure 1) of raised FC between children with longstanding CD and controls. In children with newly diagnosed CD, the median (IQR) of FC was significantly higher [36.4 (26-61) mg/kg] than in healthy controls [25.0 (23-41) mg/kg]; $p=0.045$ (Table 1) but none of these children had FC higher than 100 mg/kg (Figure 1). There was no significant correlation between FC and tissue transglutaminase antibodies ($r=0.073$; $p=0.556$) in the CD group. Similarly, for both the newly diagnosed ($r=-0.227$; $p=0.383$) children and those with longstanding CD ($r=0.145$; $p=0.360$), no associations were found between FC and the PedQL™ Gastrointestinal Symptoms Scale.

Thirteen children with newly diagnosed CD provided samples 6 months after treatment on GFD. In this group, the median (IQR) concentration of FC significantly

decreased [36.5 (26-61) vs 25 (25-25) mg/kg; $p=0.012$] and in all, but two (85%), this was below the detection limit of the assay (i.e. 25 mg/kg) at 6 month follow up (Figure 2).

DISCUSSION

There are few data on the potential clinical benefit of FC measurements in groups of individuals with chronic disease at increased risk of gastrointestinal inflammation outside IBD. Our hypothesis that children with JIA, have occult colonic inflammation was rejected, both in a treatment-naïve cohort of patients as well as in children on disease modifying treatment. FC levels were within the normal reference range in JIA children with or without increased risk of developing IBD. These findings differ from those by Stoll who observed higher concentrations of FC in children with enthesitis related arthritis(5) compared with other types of JIA and non-inflammatory conditions controls (Table 2). These opposing results may be explained by the low use of non-steroidal anti-inflammatory drugs in our participants and in accordance with previous research which suggests transient increases in FC during regular use of non-steroidal anti-inflammatory drugs(6). In our group, use of non-steroidal anti-inflammatory drugs was uncommon and intermittent. Transient increase of FC in four of our JIA patients might also be explained by concurrent subclinical gastrointestinal infection although we did not specifically look for this, at this study. Considering the findings of the current study, along with the inherent risk of IBD in this population, and early evidence which suggests that some JIA children with increased FC may suffer from subclinical IBD (7), we propose referral for further investigations only when FC remains significantly elevated in repeated measurements of FC, particularly in the absence of overt gastrointestinal symptoms.

In this study, FC was measured in two groups of patients suffering from CD; new patients at diagnosis, before and after six months on GFD, and others with longstanding disease. Our data suggest that while FC was on the whole within the normal range, its median concentration at disease diagnosis was significantly higher than in healthy controls, but decreased after six months of GFD. These results are in accordance with previous research

published by others in children and adults and is summarised in Table 2. Similar to what we have shown here, in these previous studies (8-11) no clear associations were observed between FC disease activity markers, IgA tissue Transglutaminase antibodies and FC levels (Table 2). On the bases of the findings of the current study and previous research (Table 2), we propose that FC has no value in the routine diagnostic work-up of CD. A slightly raised FC may be explained by false elevation due to mild inflammation of the rectal mucosa from nutrient malabsorption or passage of residual antigenic fragments of gliadin through the colon and rectal inflammation(12).

Conclusion

Spot measurements of FC are within normal levels in paediatric patients with JIA or CD. A significant elevation of FC in this group is not explained by the underlying diagnosis and therefore needs further investigation to exclude IBD which can occur in association with both JIA and CD.

Acknowledgments

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Figure legends

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Figure 1

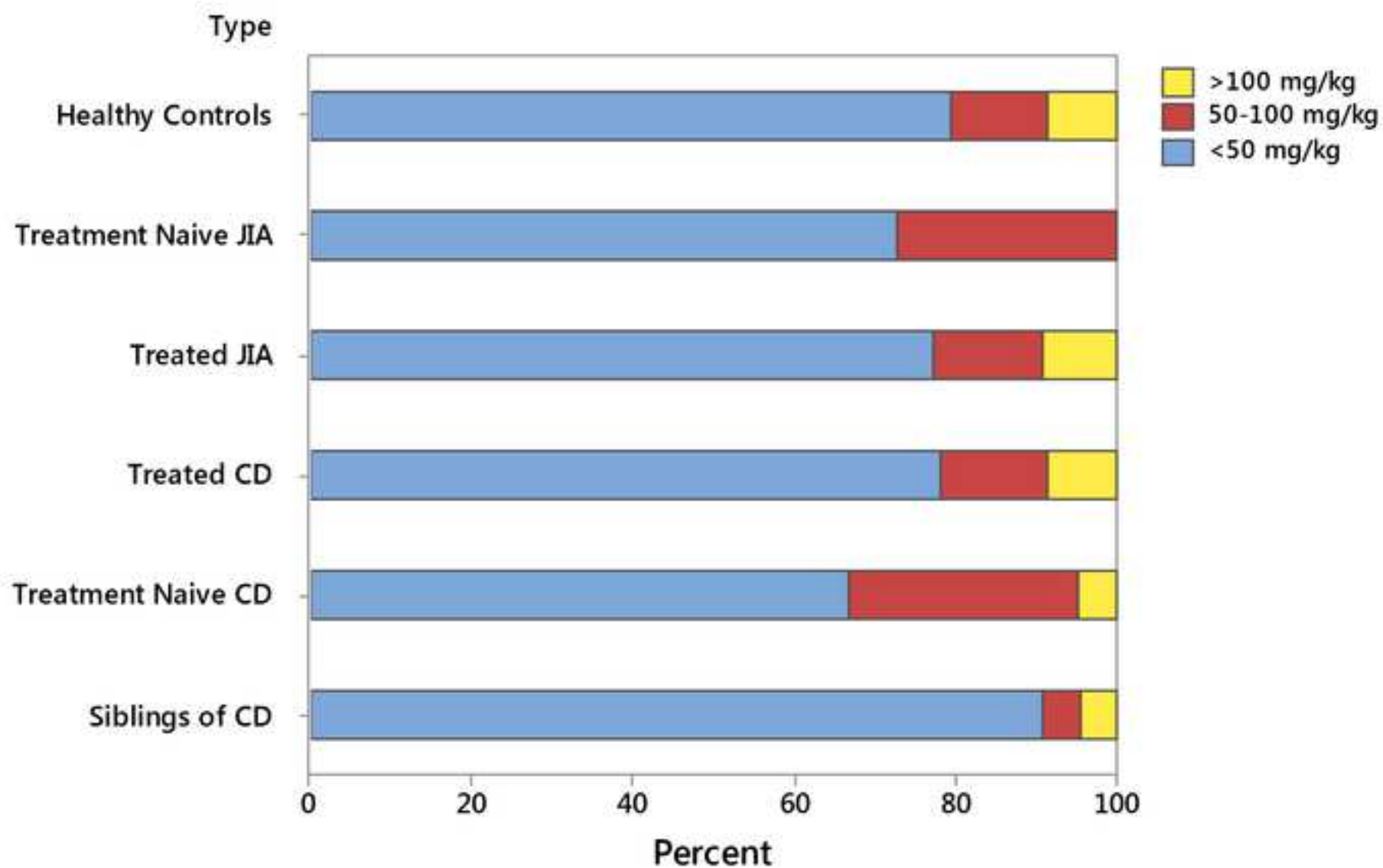


Figure 2

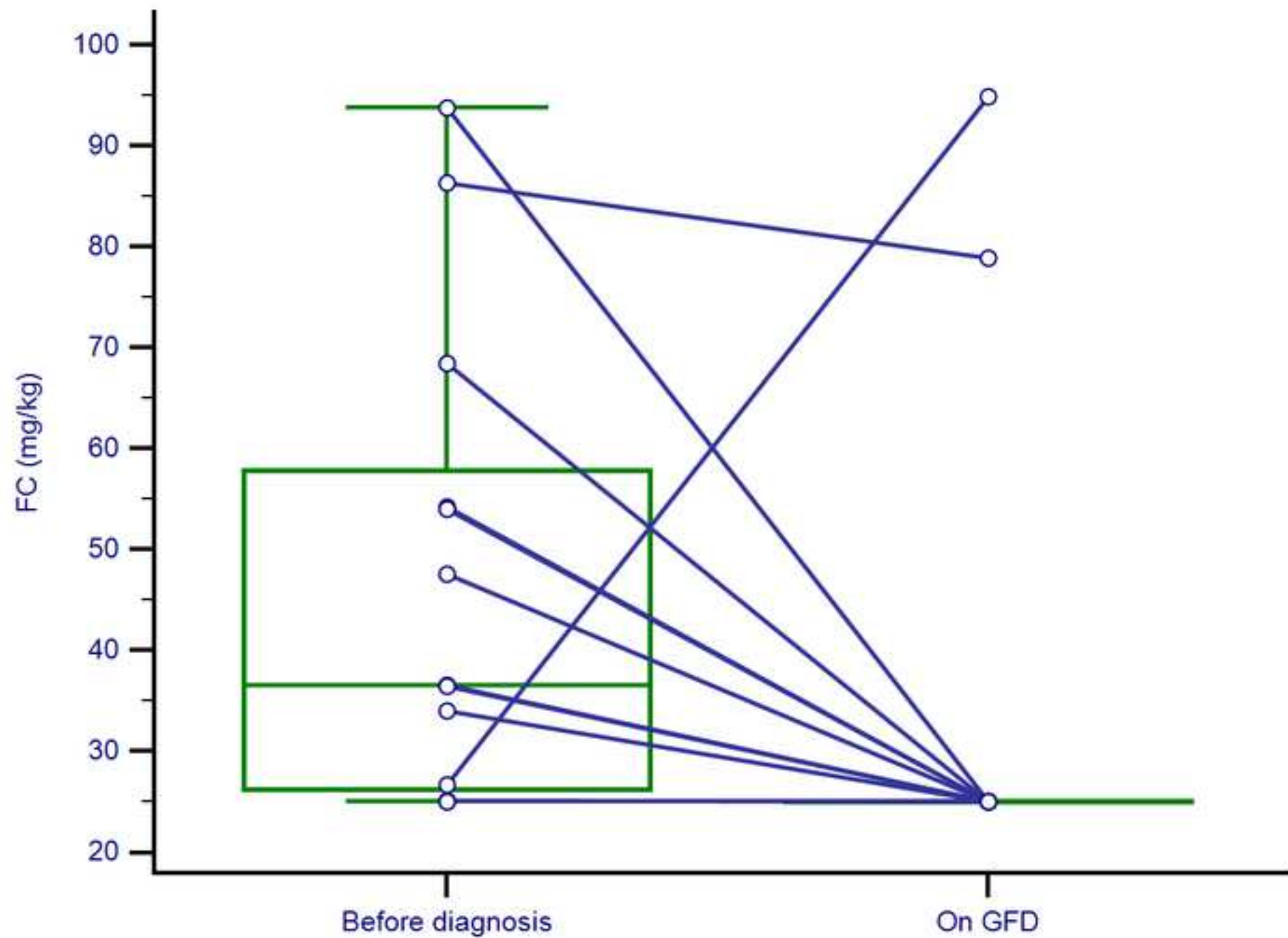


Table 1

Table 1: Demographic characteristics, and concentration of faecal calprotectin in children at risk of colonic inflammation and healthy controls

Groups	N	Gender (F/M)	Age, years (median, IQR)	FC (mg/kg) (median, IQR)
Coeliac disease				
Treated CD	46	26/20	8.9 (6.2-11.2)	25 (25-47)
<i>Raised tTG-IgA</i>	10			
Treatment naïve CD	17	9/8	10.3 (6.5-12.4)	36 (26-61)*
<i>Raised tTG-IgA</i>	17			
Juvenile idiopathic arthritis				
Treatment naïve JIA	11	5/6	5.1 (1.9-9.2)	25 (25-41)
<i>Raised CRP (>7 mg/L)</i>	2			
Treated JIA	44	29/15	10.5 (8.6-12.9)	25 (25-45)
<i>Raised CRP (>7 mg/L)</i>	4			
HLA B27+	9			25 (25-48)
Enthesitis related arthritis	13			25 (25-27)
Psoriatic arthritis	7			27 (25-56)
Parental history of IBD	4			
Other blood relatives with IBD	4			
Other				
Healthy children	92	48/44	8.3 (5.6-10.7)	25 (25-41)
Siblings of CD	22	12/10	9.0 (6.8-11.9)	26 (25-40)

* vs healthy children, p=0.045

Table 2: Evidence table of studies in faecal calprotectin in patients with coeliac disease and juvenile idiopathic arthritis

Publication	Participants	Results*	Association with FC
Capone, <i>et al.</i> 2014	Adults; NDCD: n=50, HC: n=50	FC ($\mu\text{g/g}$), HC: 45.1 (38.4), NDCD: 57.7 (29.1); p=NS Raised FC ($>75 \mu\text{g/g}$), NDCD: 10% compared with HC, 8%; p=NS	No association between FC with clinical symptoms, Marsh score, tTG
Balamtekin, <i>et al.</i> 2012	Children; NDCD: n=31 (GI symptoms n=18, no GI symptoms n=13), CD on GFD: n=33, HC n=34	FC ($\mu\text{g/g}$), NDCD [117.2 (3.2-306)] higher than HC [9.6 (1-70)] and GFD CD [3.7 (0.5-58.2)] (p<0.001) FC in NDCD with GI symptoms higher compared to no GI NDCD (p=0.04)	No association between FC with Marsh score, degree of neutrophil infiltration
Ertekin, <i>et al.</i> 2010	Children; NDCD: n=29, HC: n=10	FC ($\mu\text{g/g}$), NDCD: 13.4 (8.5), HC: 4.3 (3.3), CD GFD for 1 year: 4.6 (2.7) FC in NDCD higher than HC (p=0.004); between HC and CD GFD (p=0.8)	FC ($\mu\text{g/g}$), NDCD with total villus atrophy, 13.8 (9.3) vs partial villus atrophy, 3.7 (1.8), p=0.005
Montalto, <i>et al.</i> 2007	Adults; CD: n=28, HC: n=30	Reduction in FC levels with GFD (p=0.001) FC ($\mu\text{g/g}$), CD: 45.0 (24.2), HC: 36.5 (21.7), p=0.163; None of the participants had high FC (i.e.>100 $\mu\text{g/g}$)	No association with clinical symptoms score (p=0.92), histological score (p=0.96), neutrophil infiltration (p=0.74)
Stoll, <i>et al.</i> 2011	Children; ERA: n=9, JIA: n=17 CTD: 9, NIC: n=6	FC ($\mu\text{g/g}$), ERA: 171 (34-280), JIA: 51 (36-98, CTD: 26 (0-38), NIC: 80 (28-120) Raised FC levels was high (i.e.>121 $\mu\text{g/g}$) in proportionally more ERA patients compared to other groups (p=0.024)	No association between FC and NSAID use; One out of three patients with GI symptoms had raised FC

* Mean (SD) or median (Q1-Q3); NDCD: Newly diagnosed Coeliac Disease; HC: Healthy controls; FC: Faecal calprotectin; GFD: Gluten free diet; GI: gastrointestinal symptoms; NS: Non-statistically significant; tTG: IgA tissue transglutaminase antibodies; ERA: Enthesitis related arthritis, JIA: Other JIA, CTD: unrelated connective tissue diseases, NIC: Non-inflammatory controls, NSAID: Non-steroidal anti-inflammatory drugs